UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

June 28, 2007
TXR#: 0054628
DP Barcode No.: D340747

MEMORANDUM

SUBJECT: Endosulfan Supplemental DER and RESPONSE TO COMMENTS. The Health Effects Division's (HED) response to comments on EPA's Review of Endosulfan Developmental Neurotoxicity Study in Rats 03/15/07.

PC Code: 079401
DP Barcode No.: D327215, TXR# 0054486
MRID# 46968301, 00148264, 47155301

FROM: Judy Facey, Toxicologist 6/28/07
Reregistration Branch II
Health Effects Division (7509P)

TO: Tracy Perry, Chemical Review Manager
Special Review Branch
Special Review and Reregistration Division (7508P)

THROUGH: William Hazel, Chief for William Hazel 6/28/07
Reregistration Branch II
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I. CONCLUSIONS

The Agency has received the Endosulfan Task Force response/ comments to the Endosulfan Developmental Neurotoxicity Study in Rats, Data Evaluation Record dated 03/15/07, TXR# 0054486, and DP Barcode D327215. After additional review, the Health Effects Division retains its original conclusion for this Developmental Neurotoxicity Study.

II. ACTIONS REQUESTED

HED Developmental Neurotoxicity Committee met to discuss/evaluate the response/comments the ETF submitted to the Agency.

III. BACKGROUND

The following HED response to comments on Endosulfan was generated to address comments submitted by the Endosulfan Task Force (ETF) (MRID 47155301) to the Agency following the publication (review) of the Developmental Neurotoxicity Study Data Evaluation Record 03/15/07, TXR# 0054486, and DP Barcode D327215. It is noted that some of the comments submitted by ETF are clarification. This document is HED's response to these comments.

Response to issues raised by ETF

In the Agency's review (Anderson and Facey 2007 dated 03/15/07) of the endosulfan developmental neurotoxicity (DNT) study (Gilmore et al. 2006), it was stated that *this study is classified ACCEPTABLE and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, 83-6); OECD 425(draft).* However, the Agency's reviewer raised the following issues:

EXECUTIVE SUMMARY (page 3 of 48):
EPA: This study is classified ACCEPTABLE/Non-guideline and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, 83-6; OECD 425(draft)] due to the pending review of the positive control data.
ETF Response: Bayer CropScience has submitted positive control data for developmental neurotoxicity studies. Page 34 of the final report (MRID 46968301) notes:.....

Agency's response: The classification used is current standard practice of the Health Effects Division Developmental Neurotoxicity Committee for **all** DNT studies pending a complete systematic review of all positive control data.

1. EPA:
Unless the authors can show otherwise, it is concluded all dose levels showed a pup weight decrement in males. The male and female pup weight was not decreased during the high growth phase between birth and PND 4, but it was decreased between PND 4 and PND11.

ETF Response: The study data support the conclusion of the study director. Our analysis is contained in Appendix A. We conclude that the collective data support a NOAEL for pups of 50 ppm (3.74 mg/kg bw/day).

ETF Response: Historical control data are provided in Appendix B. There are two studies noted in which the historical control data are lower than the

body weight data for PND 11 and PND17 pups (Study 00-D72-AG and Study 00-D72-Al).

Agency's response: Customarily, historical control data are used to assess if the concurrent control data are atypical for the test species and strain used in the study under evaluation, not as a point of comparison for animals in the test group. If the concurrent control is within the range observed in the historical control data, as is the case in the endosulfan DNT study, then a comparison of the test group animals to the concurrent control is preferred. Moreover, the Agency notes that the decreases in pup weights noted at all dose levels are dose-related and occurring in both sexes further substantiating the Agency's conclusion that this is a compound-related effect. With respect to the registrant's assertion that it is not biologically plausible to have apparent discrepancies in pup weights across time periods, the Agency notes that exposure to pups varies during the lactation period particularly during the transition from exposure *via* milk only to exposure through milk and feed.

In addition, the 2-generation reproduction study (MRID # 00148264), noted a similar effect (decrease litter weight) during the lactation to weaning period in both matings in the F0 generation, which was significant at the high-dose level in the first mating and at the mid-and high dose levels in the second mating (dose-related).

IV. RESULTS/DISCUSSION/COMMENTS

After reviewing the response/comments submitted by ETF, the Health Effects Division retains its original conclusion for this Developmental Neurotoxicity Study.

Study Type	MRID	Comments
870.6300 Developmental Neurotoxicity Study-rat	46968301, 00148264, 47155301	Supplemental DER attached in review package

EPA Reviewer: David Anderson, Ph.D. & Judy Facey, Ph.D. **Signature:** Judy Facey
RRB-2, Health Effects Division (7509P) **Date:** 6/20/07
EPA Secondary Reviewer: Elissa Reaves, Ph.D. **Signature:** Elissa Reaves
RRB-2, Health Effects Division (7509P) **Date:** 6/22/07

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SUPPLEMENTAL
DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat;
OPPTS 870.6300 (83-6); OECD 426 (draft)

PC CODE: 079401 **DP BARCODE: D340747**

TEST MATERIAL (PURITY): Endosulfan |99.1% pure||6,7,8,9,10,10-hexachloro-1,5,5a,9,9a-hexahydro-6,9-methano-2,4,3,-benzodioxathiepin-3-oxide|

CITATION: Gilmore, R.; Sheets, L.; Hoss, H. (2006) A Developmental Neurotoxicity Study with Technical Grade Endosulfan in Wistar Rats. Project Number: 201563, 05/D72/YF. Dated September 26, 2006. Unpublished study prepared by Bayer Corp. 1062 p. MRID# 46968301

Volger, B. (2007) Endosulfan: Developmental Neurotoxicity Study in Rats (MRID 46968301) Response by the Endosulfan Task Force (ETF) to issues raised in EPA's Data Evaluation Record (DP Barcode D327215). Project Number: D327215. Unpublished study prepared by Endosulfan Task Force. 40 p. MRID# 47155301

SPONSOR Endosulfan Task Force

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46968301, 47155301), endosulfan (99.1% a.i. batch# EGPC400349) was administered to 30 female Wistar Crl:WI (Han) rats per group in the diet at dose levels of 0, 50,150, or 400 ppm or 0, 3.74, 10.8 or 29.8 mg/kg bw/day from gestation day 6 through postnatal day 21; doses were adjusted to nearly constant mg/kg/day throughout lactation. Dams were evaluated for body weight, food consumption, clinical observations, and FOB on GD 13 and 20. Offspring were dosed only through nursing mothers and when eating the mother's diet. Subsets of dams with acceptable litters were evaluated for FOB on LD 11 and 21. Selected offspring from each group were evaluated using detailed clinical observations, body weight, food consumption, developmental landmarks for sexual maturation, automated measurements of activity in a figure eight maze, auditory startle habituation, learning and memory [passive avoidance and a water maze task]. Ophthalmic examinations were conducted. Tissues were collected for morphometry and microscopic examination on PND 21 [brain] and at study termination [brain, an assortment of neural tissue, and skeletal muscle]. Sperm analysis was performed on testes and epididymal sperm.

Body weight of dams was statistically significantly decreased at all dose levels on gestational day [GD] 13 and 20 and lactational day [LD] 0, 4 and 7 at the two highest dose levels. However, when this body weight decrease was correlated with food consumption data, food efficiency calculations suggested that a decreased food consumption from an unpalatable diet may have caused the body weight decrement at the LDT and the MDT. This was more certain at the LDT, but less definitive at the MDT. Only the HDT induced adverse body weight decrement when combined with food efficiency. No other effects were noted in dams. It is noted that a 90-day neurotoxicity study [MRID 46444401] in Wistar rats did not show body weight decrement in non-pregnant females at the HDT of 45.5 mg/kg/day. However, food consumption in these females was decreased statistically at the 16.6 and 45.5 mg/kg/day during the first week of the 90-day study.

The NOAEL for dams is 3.7 mg/kg/day. The LOAEL is 10.8 mg/kg/day based on decrease body weight, food consumption and food efficiency.

Pup weight on a litter basis was decreased in males and females [males:8.3% - 13.2%, $p<0.01$; females: 8.0%-13.7%, $p< 0.01$] in all dose levels on PND 11 and PND 17 [males: 6.9%-8.8%, $p<0.05$] in males only. The male and female pup weight decrement at all dose levels at PND 11 was reflected in a decrement in the pup weight gain from PND 4-11. Although statistically significance occurred in data on male weight gain PND 11-17, this pup weight gain decrement is believed to be due failure to regain weight lost during the decrement from PND 4-11. No pup weight decrement was seen at birth or PND 4 at the LDT. Since it is unknown whether the pup weight decrement was due to unpalatable endosulfan in the mother's milk, a reduced milk supply or a toxic effect of endosulfan in the milk, it will be assumed to be a toxic effect until data are submitted to show otherwise.

Sexual development was delayed as shown by preputial separation in males at the MDT and HDT of [47.1 and 46.8 days, respectively compared with 44.9 days in controls] and delayed vaginal opening in females at the LDT [34.2 days] and MDT [34.2 days] and at HDT [34.0 days] compared with control at 33.0 days. The delayed preputial separation in males had no effect on sperm parameters measured at termination. The vaginal opening may have been incidental since the data were not dose related and within historical control range [32.0 to 34.6 days]. There was no difference in body weight among dosed groups and controls at the time of measurement.

No definitive effects were seen in the FOB with offspring. However, 1/16 and 3/16 PND 4 male pups and 0/16 and 2/16 PND 4 females showed minimal resistance to removal with minimal vocalization at the MDT and HDT, respectively. Other groups tested at PND 11, 21, 35 or 60 showed this effect in 0/16 offspring tested. PND 21 male pups showed dose related slight increased rearing [$p<0.05$] at the HDT and PND 21 female pups showed increased rearing at the MDT [6.4, $p< 0.05$] and HDT [5.5, $p>0.05$] only.

No effects were shown when tested for acoustical startle, learning and memory in the passive avoidance or water maze tests, which showed habituation.

Perfused fixed brain weight in PND 21 male pups was reduced 5% at the HDT, but the body weight ratio was nominally increased. Brain weight for fixed perfused PND 21 and PND 75 females was unchanged as well as fixed perfused and non-perfused PND 75 male brain weight. Morphometric analysis of the female brains showed a decrease in hippocampal gyrus of 0.158 mm [a 10% decrease, $p<0.05$ compared with controls] at the HDT only.

There was no NOAEL for pups. The LOAEL was the LDT at 3.74 mg/kg/day based on decreased pup weight at PND 11 and decreased weight gain at PND 4-11. At the MDT, possible delayed preputial separation in males occurred. No neurotoxic effects were seen at

the LDT or MDT. Possible effects were shown at the HDT for PND 21 male rearing in the FOB [within historical control range], PND 21 male fixed perfused brains and a 0.158 mm statistically significant decrease in female morphometric measurements on the PND 21 hippocampal gyrus at the HDT, the only dose level measured [control 1.623 mm, HDT 1.469 mm, $p<0.05$], but these values in control and at the HDT were within historical control range of 1.38 to 1.69 mm. These possible neurotoxicity-related effects could be biologically significant and treatment related at higher doses, but are not definitively shown in this study at the HDT.

This study is classified as **Acceptable/Non-guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] due to the pending review of the positive control data.

COMPLIANCE: Signed and dated GLP Quality Assurance, and Data Confidentiality statements were provided

CONCLUSION: The Agency has received the Endosulfan Task Force response/ comments to the Endosulfan Developmental Neurotoxicity Study in Rats, Data Evaluation Record dated 03/15/07, TXR# 0054486, and DP Barcode D327215. After additional review, the Health Effects Division retains its original conclusion for this Developmental Neurotoxicity Study (MEMO dated 06/28/07, TXR# 0054628, and DP Barcode D340747).

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Chemical: Endosulfan

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